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An Outbreak of Post-Vaccinal Rabies (*Rage de Laboratoire*) in Fortaleza, Brazil, in 1960

Residual Fixed Virus as the Etiological Agent *

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The repeated isolation of fixed rabies virus from the CNS tissues of victims of an acute and lethal outbreak of encephalomyelitis in Fortaleza, Brazil, in November 1960, following vaccination with a locally produced killed-virus anti-rabies vaccine of the Fermi type is considered as definitive evidence of the rabic etiology (vaccinal fixed-virus rabies, rage de laboratoire*) of this outbreak. Eighteen persons were affected, all of whom died.*

The clinical picture of paralytic rabies was recognizable in all of these 18 patients. The well-marked characteristics of an acute infection permit the easy differentiation of the paralysis caused by fixed rabies virus from post-vaccinal accidents that occur as allergic reactions.

The incriminated anti-rabies vaccine was found to contain fixed live rabies virus at a titre of $10^{-3.0}$. After one year of storage under refrigeration, the vaccine still contained fixed rabies virus, at a titre of $0{,}2 \times 10^{-1.0}$.

Subsequent laboratory studies tend to indicate that the curve of inactivation of fixed virus by phenol does not follow a linear function but rather resembles the curve of inactivation of poliomyelitis virus by heat and formol according to the Salk technique. It is suggested that the antigenicity of the so-called "killed-virus" anti-rabies vaccines is actually due to the presence in them of residual amounts of live virus.

Post-vaccinal rabies, which is the subject of the present report, has been a subject of controversy among specialists in rabies ever since C. F. M. Peter made impassioned charges against Louis Pasteur before the Academie de Médecine in Paris in January 1888 (Remlinger, 1935a, 1935b, 1952; Blood, 1950). Unfortunately, these debates have confused rather than clarified the issue.

Elsewhere, my colleagues and I have reported in detail upon the circumstances that surrounded the outbreak of laboratory rabies infection (*rage de laboratoire*) which occurred in the city of Fortaleza, in the State of Ceará, Brazil, in 1960 (Pará, Passos & Bezerra, 1964). The purpose of the present report is to summarize the clinical and epidemiological aspects of this outbreak, to report upon the investigation that my colleagues and I made concerning its etiology, and to comment upon this incident from both the scientific and the public health points of view.

POST-VACCINAL RABIES ENCEPHALOMYELITIS IN FORTALEZA, BRAZIL, IN 1960

Geography and population

The city of Fortaleza is situated within the tropical zone on the northeastern coast of Brazil and is the capital of the State of Ceará. The city has about 400 000 inhabitants and a rather large canine population.

Rabies situation

Rabies may be considered as endemic in Fortaleza, the principal vector and victim being the dog, with only sporadic human cases. As yet, the public

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health authorities have not initiated a programme to eliminate street dogs and to vaccinate the remainder of the dog population. The local public health department does, however, maintain a laboratory that produces rabies vaccine for use exclusively in the treatment of humans. Unfortunately, this laboratory operates under unsatisfactory technical conditions. This situation is not uncommon in the other States of Brazil and in many other localities in South America.

Post-vaccinal accidents in 1960

Occurrence. Between 11 and 24 November 1960, the Rabies Vaccination Centre of Fortaleza reported 18 cases of a febrile disease with paralytic complications and rapid death in vaccinated persons. All of these individuals had been recently bitten by street dogs and all of them were therefore receiving anti-rabies treatment with a vaccine that had been produced recently by the local public health laboratory that has been mentioned.

Inasmuch as these fatalities had occurred only in vaccinated persons, the health authorities of the State of Ceará suspected that the vaccine was incriminated and decided to withhold its further use. They also appealed to the Federal Department of Public Health to investigate the matter and to assist the local authorities in controlling the outbreak. Our Virology Laboratory at the Osvaldo Cruz Institute in Rio de Janiero was requested to perform the necessary laboratory studies.

Clinical observations. While we in the Virology Laboratory were given only piecemeal information concerning the cases under investigation, the similarities of these cases were such that we were able to construct an over-all clinical picture.

The characteristic course of the disease was as follows. After a short prodromal phase that lasted for only one or two days, with general malaise, headache, rachialgia, neck pains, and poorly defined muscular pains, the disease declared itself by an elevated and continuous fever and very intense headache (16 cases), agitation and convulsions (6 cases), paresis (14 cases), and muscular spasms. These early symptoms were followed by frequent projectile vomiting (13 cases), dysphagia, diplopia, ascending paraplegia, restlessness, muscular tremor (14 cases), and urinary incontinence. Finally, with the patient in a state of obnubilation, torpor set in (9 cases), followed within two to nine days by coma and death.

Epidemic data. Of a total of 66 individuals who were treated with the suspected rabies vaccine, 18 experienced an acute encephalomyelitis, with associated fever, that invariably terminated fatally. The following data were ascertained from these 18 cases.

1. Each of the 18 patients had been bitten by a street dog.

2. Of the 18 dogs involved in these incidents, only five were found to be actually rabid, and seven were found to be healthy. (The histories of the remaining six animals were unknown, so they were classified as being suspected of rabies.)

3. The outbreak of human rabies was confined to persons who had been vaccinated; there were no cases among the contacts of the 18 victims.

4. In nine of these 18 cases, vaccination had been initiated within 24 hours after the patient had received a dog bite, including five cases in which the initial treatment had been received on the same day as the bite. In two cases, vaccination was started within two days, in five cases within three days, in another case within five days and in the last case within eight days.

5. The period of incubation ranged between 4 and 13 days, counting from the administration of the first dose, with a mean of eight days.

6. The disease was progressive and acute, and death occurred between two and nine days (average of five days) from the time of onset.

7. Most (14) of the 18 victims received three or more doses of the suspected vaccine, either daily or on alternate days. Two others received two doses, and two received only one dose.

8. Nine patients were males, and nine were females.

9. While the distribution of these patients by age clearly showed the predominance of individuals aged less than 20 years (10 of the 18 cases, the remainder being 30 or under), this finding probably corresponds to the usually greater frequency of dog bites, and consequently of anti-rabies vaccination, in children and adolescents in urban areas.

Pathological findings. Material for study from three of the cases in the post-vaccinal outbreak was received in our laboratory. This material included specimens of the brain, cerebellum, pons, and cervical cord, preserved in 10% formol-saline solution. The lesions that were found in these

tissues permitted the making of an over-all description, taking into account the variations in intensity, as follows.

1. The brain tissue showed an acute inflammatory reaction, focal necrotic degeneration, with congestion, perivascularitis, gliosis, chromatolysis, and neurolysis, as well as satellitosis and neuronophagia of the motor neurons. Negri bodies were absent, and no areas of demyelinization were seen.

2. The samples of cerebellar tissue showed discrete involvement of the Purkinje cells and a moderately diffuse lymphocytic infiltration. Again, no Negri bodies were seen.

3. Tissue from the pons showed lesions that were very similar to those found in the cortical tissue. Panmyleitis was encountered in the cord tissue, being more intense in the grey matter. Neither Negri bodies nor areas of demyelinization were found in tissue from the pons or cervical cord.

On the basis of these histopathological findings, the following diagnosis was reached: an acute viral encephalomyelitis similar to that which is produced by a fixed rabies virus.

Suspected pathogen. As stated above, the anti-rabies vaccine that had been used in these 18 patients after they had received dog bites was of local provenance and had been prepared according to the Fermi technique; that is, by the utilization of phenol alone, in a concentration of 0.5%, to inactivate the virus. The final concentration of this vaccine was 5% nervous tissue. The vaccine had been prepared from sheep brains with a fixed rabies virus of the original Pasteur strain, using material that had undergone 2024 passages in rabbit brain. (This material had been received recently from a federal veterinary research institution.) The vaccine was released for use (4 November 1960) in humans on the fourth day after its preparation, with no precautionary measures other than bacteriological sensitivity tests. Consequently, this vaccine was not held for the time recommended by the approved international standards. Four vials of this material were received by our laboratory, and in all of them it was possible to isolate fixed virus, both in rabbits and in Swiss white mice.

Etiology. A detailed account is given below of the neurotropic virus that was isolated from the suspected vaccine, from the central nervous system tissue samples from the three human cases mentioned above (preserved at low temperature or in glycerol), and from the rabbit brain material that was the source of virus used in the preparation of the vaccine.

Altogether, seven isolations of the virus were made in the brains of rabbits or of Swiss mice from the material that had been received for etiological investigation. The behaviour of the isolated virus as well as the experimental pathological findings and the immunological characteristics encountered led us to an identification of fixed rabies virus in all seven instances (see the table). The grounds for this identification are given below.

1. In rabbits, Swiss mice and guinea-pigs, the experimental infection showed, without exception, a period of post-inoculation incubation of only 4-7 days.

2. In the guinea-pig and the rabbit, the experimental infection produced by each of the seven isolated viruses was typical of paralytic rabies.

3. The histopathological findings in the central nervous systems of the inoculated animals corresponded to those of fixed rabies virus encephalomyelitis.

4. Despite careful search, Negri bodies were not found in any of the CNS material that was taken from the three human cases.

5. The first passage in Swiss mice from any of the seven virus samples that were isolated invariably was shown to be of the order of $10^{-5.0}$ to $10^{-7.0}$.

6. The presence of rabies was definitely demonstrated immunologically by means of serum neutralization tests, cross-immunity tests using a reference strain of fixed rabies virus, and by complement-fixation studies using an antigen prepared according to a technique similar to that described by España & Hammon (1947, 1948). The cross-immunity and serum-neutralization tests were conducted in accordance with the recommendations of d'Antona et al. (1954).

DISCUSSION

A bacteriologically sterile anti-rabies virus of the Fermi type that had been maintained at $\pm 10°C$ since the time of its manufacture one month previously was shown to contain, when injected into laboratory animals, viable and virulent fixed virus in a dilution of $10^{-3.0}$. After being kept under refrigeration at a uniform temperature for an entire year, the vaccine still showed the presence of fixed virus in a dilution of $0.2 \times 10^{-1.0}$.

CHARACTERISTICS OF VIRUS STRAINS ISOLATED AFTER POST-VACCINAL RABIES ACCIDENT

Virus strain	Source	Pathogenicity			Pathology		Immunity			
		Experimental animal	Result [a]	LD_{50} [b]	Lesions	Negri bodies	Homologous	Heterologous	Neutralization test [c]	Complement-fixation test
AG, H.A., H.3	Brain or cerebellum (fatal human cases)	White mouse (Swiss); Rabbit Guinea-pig	Paralytic rabies	$10^{-5.0}$ to $10^{-7.0}$	Fixed-virus encephalomyelitis	None	−+	+	+	+
MJ, ARE	Suspected vaccine (original vials)	Guinea-pig	Paralytic rabies	$10^{-5.0}$ to $10^{-7.0}$	Fixed-virus encephalomyelitis	None	+	+	+	+
Co.2024, Ce. 1	Rabbit brain passage (source of virus)	Guinea-pig	Paralytic rabies	$10^{-5.0}$ to $10^{-7.0}$	Fixed-virus encephalomyelitis	None	++	+	+	+

[a] In white rabbits, weighing ca 2 kg, the incubation period is 5-8 days.
[b] Determined in Swiss white mice 30 days old through cerebral inoculation.
[c] Against a stock hyperimmune anti-rabies serum.

When injected into man intramuscularly or subcutaneously in the deltoid region four days after its manufacture, this virulent vaccine produced encephalomyelitis in 18 of the 66 persons who were vaccinated with it. This infection proved to be fatal in all cases.

The virus that was isolated from the incriminated vaccine, as well as from the CNS tissue of three patients who died, was identified as fixed virus. None of these samples exhibited any remarkable features either in pathogenicity or in immunological behaviour.

Means for the recognition of the post-vaccinal rabies that is caused by fixed virus and is called, for lack of a better term, laboratory rabies (*rage de laboratoire*) have been available since the definitive studies of Remlinger (1935a, 1935b, 1952) and of Remlinger & Bailly (1938). The following diagnostic criteria, which were originally established by Remlinger and later confirmed by other investigators in several countries and followed by ourselves in the present study, may be laid down as described below.

1. An acute post-vaccination accident occurs within one month after the initiation of anti-rabies treatment.

2. The clinical picture is of a febrile encephalomyelitis, generally exhibiting an ascending paralysis of the Landry type. In such cases, a flaccid paralysis of the limbs is noted.

3. Neither furious excitement nor true hydrophobia is seen.

4. Death results from bulbar paralysis.

5. The pathological findings are specific for fixed rabies virus infection.

6. Fixed rabies virus is isolable from the CNS of the patient, especially from his medulla. Indeed, the isolation of fixed virus from the medulla is considered to be the *sine qua non* of a positive diagnosis of laboratory rabies.

According to Remlinger (1935a, 1935b, 1952), laboratory rabies is caused by vaccinal inoculation. While it is fortunately true that these accidents occur but rarely, it has been found that they are three times more frequent when live, attenuated vaccines, such as those of Pasteur, Hoegyes or Puntoni are used than when vaccines of the killed-virus type, such as those of Fermi, Semple, or Peck are used.

Laboratory rabies can be included among the neuroparalytic accidents that are associated with anti-rabies treatment, but it should be distinguished from authentic cases of rabies that occur after vaccine therepy in individuals in whom the immune response has failed to develop. Laboratory rabies therefore constitutes a specific neuroparalytic accident that could, beyond all doubt, be prevented by

the adoption of control measures that would permit the release for use of only potent and safe vaccines for either human or veterinary use. Today, the occurrence of laboratory rabies must be considered, from the aspect of medical ethics, as the result of unpardonable negligence.

It should be noted here that, as well as laboratory rabies, there are other accidents that are related to routine anti-rabies treatment. These may be summarized as follows: local and systemic allergic reactions to the vaccine; post-injection shock, with syncopal manifestations; neuritis and neuralgia, most commonly affecting the facial and ocular motor nerves; lumbodorsal myelitis, with muscular paresis and spasms or atony of the sphincters; ascending encephalomyelitis of the Landry type, with or without bulbar paralysis; and a post-vaccinal encephalitis similar to that found in vaccinia.

There is a common denominator to these other neuroparalytic accidents that are provoked by anti-rabies vaccine derived from nervous tissue and which well might be termed non-specific; namely, that usually there is complete recovery. A few victims of these accidents suffer permanent after-effects, however, and the mortality rates range between 10% and 30% only.

The protracted evolution and varying nature of the symptoms shown by the victims of these accidents, which may or may not include manifestations of infection, relate these accidents to the encephalitides as well as to multiple sclerosis.

The allergic nature of these non-specific anti-rabies vaccine accidents is conditioned by a sensitization of the individual to the nervous tissue or, more exactly, to the " paralysing factor " which is chemically related to myelin. The anatomical basis of this reaction is demonstrated by the occurrence of diffuse demyelinating lesions that produce encephalomyelitis or impairment of the ganglia and nerve roots (Bassoe & Grinker, 1930; Marinesco & Draganesco, 1938; Uchimura & Shiraki, 1957). Experimental evidence of the allergic nature of these accidents was first offered by Rivers, Sprunt & Berry (1933) and subsequently confirmed by Jervis (1954).

At this point, we suggest that the findings of the present investigation of an outbreak of laboratory rabies raise the suspicion that the antigenicity of some of the so-called " killed virus " anti-rabies vaccines is probably due to the presence in them of trace amounts of living virus. Studies that are in progress in our laboratories already allow us to predict that, as was to be expected in view of the physicochemical nature of the material that is to be inactivated by phenol and by heat, the curve of inactivation of the rabies virus does not follow a linear function; on the contrary, it resembles the curve of inactivation by heat and formol of the poliomyelitis virus by the Salk technique (Gard, 1955; Schultz et al., 1957).

We are convinced that some inactivated fixed-virus vaccines may possess poor immunogenicity and produce some undesirable side-effects that would limit their prophylactic use in man or in animals. If this is indeed the case, another vaccine derived from an avirulent strain must be sought actively, so that human prophylaxis against rabies may be developed along more precise and scientific lines than in the past, and that real and safe protection against rabies may be provided, especially in populations that are particularly exposed.

RÉSUMÉ

La rage est endémique chez les chiens à Fortaleza, Brésil, avec quelques cas humains sporadiques. Les auteurs étudient 18 cas de « rage de laboratoire » apparus dans cette ville, en 1960, parmi 66 personnes en traitement par un vaccin local type Fermi. Toutes avaient été récemment mordues dans la rue. Des 18 chiens en cause, 12 furent retrouvés, parmi lesquels 5 seulement étaient rabiques. La maladie n'apparut que chez des personnes vaccinées et aucun contact des victimes ne présenta de troubles. Le vaccin, préparé sur cerveau de mouton, à partir d'un virus fixe provenant de la souche Pasteur originale, était à la concentration de 5% de tissu nerveux dans du formol à 0,5%. Il avait été délivré quatre jours après sa préparation, sans autres précautions qu'un contrôle bactériologique. Le laboratoire isola de chacun des quatre flacons de ce vaccin qui lui furent confiés un virus fixe qui se développa chez le lapin et la souris blanche. Les victimes avaient reçu de une à trois doses et plus de ce produit, entre le jour de la morsure et le huitième jour après celle-ci. L'incubation dura quatre à treize jours, à partir de la première dose. Après une phase prodromique de un à deux jours avec malaise général, céphalée, rachialgie, douleurs musculaires mal définies, s'installaient une fièvre élevée continue et une violente céphalée (16 cas), avec agitation et convulsions (6 cas), des parésies (14 cas) et des spasmes musculaires.

Ces signes précoces furent suivis de vomissements en fusée, de dysphagie, diplopie, paraplégie ascendante, agitation, trémulations musculaires et incontinence d'urine. L'évolution se fit d'une obnubilation à un état de torpeur, puis un coma de deux à neuf jours terminé par la mort. L'examen histopathologique trouva des lésions neuro-cérébrales semblables à celles de l'encéphalite provoquée par le virus fixe, d'intensité variable mais de type constant: inflammation aiguë avec foyers nécrotiques, infiltration lymphocytaire diffuse et modérée au niveau du cerveau et du cervelet, panmyélite touchant surtout la substance grise; on ne trouva pas de corps de Negri.

Cette rage post-vaccinale (rage de laboratoire) reproduit la description classique de Remlinger. Le virus isolé aussi bien du vaccin incriminé que du tissu nerveux de trois cas a été identifié comme le virus fixe, sans caractères pathogéniques ou comportement immunologique particuliers. Une rage apparaissant après échec de la vaccination est à éliminer. La rage de laboratoire appartient aux accidents neuro-paralytiques du traitement antirabique, accidents spécifiques que des mesures de contrôle des vaccins à usage humain ou vétérinaire doivent supprimer. Le vaccin en cause, après un mois, contenait des virus vivants à un titre de $10^{-3,0}$ et, après un an, de $0,2 \times 10^{-1,0}$, et sa courbe d'inactivation par le formol et la chaleur doit ressembler à celle du vaccin antipoliomyélitique préparé par la technique de Salk. L'auteur souhaite qu'une souche avirulente soit recherchée pour permettre la préparation d'un vaccin actif et sans danger.

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